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In Vitro Activity of the Spermicide Nonoxynol-9 Against *Chlamydia trachomatis*

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The in vitro activity of nonoxynol-9 against four serotypes (C, D, H, and K) of *Chlamydia trachomatis* was investigated. A constant inoculum of each serotype was exposed to serial twofold dilutions (1:100 to 1:800) of Koromex, Conceptrol, or reference preparations (not containing nonoxynol-9) for 4 and 24 h at 37°C. The mixtures of nonoxynal-9 or nonnonoxynol preparations and control inocula were dispensed into triplicate wells containing McCoy cell monolayers. After incubation at 37°C, the monolayers were fixed and stained with idoine and examined for evidence of infection with *C. trachomatis*. All nonoxynol-9-containing preparations showed marked antichlamydial activity as judged by percent reduction of glycogen-containing intracytoplasmic inclusions. The reference preparations, which did not contain nonoxynol-9, were markedly less active when tested in this in vitro system.

Nonoxynol-9 (N-9), a membrane-active detergent, is the active ingredient in numerous commercially available intravaginal contraceptive foams, inserts, and jellies. Previous studies have shown that this compound is active in vitro against many pathogens causing sexually transmitted diseases. However, the activity of N-9 against *Chlamydia trachomatis* has not been well studied. In this study, we investigated the in vitro activity of N-9 against *C. trachomatis* by using two commercially available spermicides, both containing N-9, and reference preparations not containing N-9.

MATERIALS AND METHODS

Microorganisms. Four strains of *C. trachomatis*, serotypes C, D, H, and K, were tested. All strains were provided by E. R. Alexander, Atlanta, Ga., and were selected because they are representative of the strains commonly associated with urethritis in men and cervicitis in women.

Contraceptives. The contraceptive products used in this study were the commercial preparations Conceptrol (Ortho Pharmaceutical Laboratories, Raritan, N.J.), containing 4% N-9, and Koromex (Youngs Drug Products, Piscataway, N.J.), containing 2% N-9. Although each contained N-9 as the spermicidal agent, the contraceptives differed slightly with regard to other constituents. Reference formulations of each product were provided by the manufacturers and were identical to the commercial formulations except for the absence of N-9.

Procedures. McCoy cells were prepared in 75-cm^2 tissue culture flasks and harvested after 7 days of growth. The cells were trypsinized and dispensed into 96-well microtiter plates to yield a density of 10^4 cells per well. Plates thus prepared were used within 4 days.

Studies were performed to assess the possible toxic effects of spermicides and reference preparations on McCoy cells. Serial dilutions of spermicides and reference preparations (1:10 to 1:2,560) were prepared in cell culture media. These dilutions were then dispensed into 96-well microtiter plates that contained McCoy cell monolayers and were incubated for 48 h at 37°C. Cellular toxicity was assessed by two methods: (i) visual determination of the condition of the monolayer under ×100 magnification and (ii) the ability of the cells to exclude trypan blue.

The antichlamydial activity of N-9 was assayed by the inoculation of serial twofold dilutions (1:100 to 1:800) of spermicide (N-9) or reference (non–N-9) preparations, mixed with a constant inoculum of each of four strains of *C. trachomatis*, into McCoy cell monolayers. Each of these mixtures and controls of cell culture media and inocula of the four strains had been incubated at 37°C for 4 and 24 h. Before McCoy cells were exposed to mixtures of *C. trachomatis* with either N-9 or non–N-9 preparations and control inocula, culture media were removed by aspiration, and the cells were treated with 0.1 ml of DEAE-dextran (30 μg/ml in 0.85% saline) for 30 to 60 min. After the DEAE-dextran was removed, the cells were exposed to the above serial twofold dilutions of the mixtures of N-9 or non–N-9 preparations and inocula of *C. trachomatis*. These dilutions and control inocula of each strain of *C. trachomatis* were dispensed into triplicate wells. Microtiter plates were then centrifuged at room temperature for 1 h at 600 × *g*, and the supernatant was removed from each well. Next, 0.1 ml of media containing 0.5 μg of cycloheximide per ml was added to each well, and the cells were incubated at 37°C for 48 h in a CO_2 incubator. McCoy cells were then fixed and stained by the following three-step method: (i) cells were fixed with a methanol-formalin mixture for 10 min, and the solution was removed; (ii) the cells were fixed with absolute methanol for 10 min, and the methanol was removed; (iii) then the cells were stained with Jones iodine for 10 min. After removal of the iodine, the cells were covered with equal parts of Jones iodine and glycerol and examined for intracytoplasmic inclusions. The antichlamydial activity of the N-9 was determined by microscopic examination of the McCoy cell monolayers,

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TABLE 1. Antichlamydial activity of conceptrol and koromex spermicides

Duration of incubation (h)	Antichlamydial agent	*C. trachomatis* serotype	% Reduction in no. of chlamydial inclusions relative to controls[a] at agent dilution:			
			1:100	1:200	1:400	1:800
4	Conceptrol (with N-9)[b]	C	100	100	83	70
		D	100	100	78	34
		H	100	100	50	17
		K	100	89	0	0
4	Conceptrol reference (without N-9)	C	40	0	0	0
		D	43	35	0	0
		H	67	12	0	0
		K	25	0	10	0
4	Koromex (with N-9)[b]	C	100	72	10	0
		D	100	75	25	0
		H	100	100	91	42
		K	100	0	0	0
4	Koromex reference (without N-9)	C	0	0	0	0
		D	0	0	0	0
		H	0	0	0	0
		K	0	0	0	0
24	Conceptrol (with N-9)[b]	C	100	100	100	94
		D	100	100	0	0
		H	100	100	96	69
		K	100	97	65	27
24	Conceptrol reference (without N-9)	C	45	0	0	2
		D	44	32	2	0
		H	54	29	7	3
		K	25	20	0	0
24	Koromex (with N-9)[b]	C	100	100	47	0
		D	100	69	18	0
		H	100	86	79	18
		K	100	68	0	0
24	Koromex reference (without N-9)	C	0	0	0	0
		D	7	0	0	0
		H	0	11	7	7
		K	0	0	0	0

[a] Mean counts of intracytoplasmic inclusions in three control wells (4 h): C, 500; D, 35; H, 60; K, 400. Mean counts (24 h): C, 500; D, 41, H, 140; K, 375.
[b] Concentrations of nonoxynol-9 by dilutions: Conceptrol—1:100 (40 μg/ml), 1:200 (20 μg/ml), 1:400 (10 μg/ml), 1:800 (5 μg/ml); Koromex–1:100 (20 μg/ml), 1:200 (10 μg/ml), 1:400 (5 μg/ml), 1:800 (2.5 μg/ml).

counting glycogen-containing intracytoplasmic inclusions. This was done under ×400 magnification, using a Leitz-Diavert inverted microscope (Rockleigh, N.J.).

RESULTS

Incubation of N-9 for 48 h with McCoy cells was found to affect the monolayers. This effect was of two types. (i) Toxicity manifested by the appearance of granulation within the cytoplasm in N-9 dilutions up to 1:320. This was not observed in the non–N-9 dilutions. (ii) Toxicity manifested by the inability of the cells to exclude trypan blue was also observed. The latter effect was seen in McCoy cells treated for 48 h with N-9 preparations at all dilutions. McCoy cells treated with non–N-9 formulations were able to exclude trypan blue at every dilution. Although N-9 did have some toxic effects on McCoy cells after 48 h of exposure and at higher spermicide concentrations, the effect was minimal after 1 h of exposure. Infectivity of *C. trachomatis* was believed to be preserved in this system since intracellular replication took place in spite of the initial 1-h exposure to N-9 in the original inoculum.

The results of the studies of N-9 antichlamydial activity are presented in Table 1. At higher concentrations, there were marked differences in activity against *C. trachomatis* by N-9 and non–N-9 preparations. N-9 preparations were very inhibitory at dilutions of 1:100 and 1:200 as judged by observed reductions in intracytoplasmic inclusions relative to the number of inclusions in control wells. At the highest concentration (1:100), N-9 preparations caused a 100% reduction in the number of inclusions for all strains at both 4 and 24 h of incubation. The non–N-9 Conceptrol effected moderate reductions (25 to 67%), whereas the non–N-9 Koromex showed virtually no activity. A dilution of 1:400 was required to render the non–N-9 Conceptrol inactive. The N-9-containing Conceptrol was generally more active than the N-9 Koromex, especially after 24 h of incubation.

DISCUSSION

Sexually transmitted infections due to *C. trachomatis* are a major cause of disease in the United States and other industrialized nations. These infections are now more common than those owing to *Neisseria gonorrhoeae* (9, 11). Both the tendency for these infections to produce few symptoms and the lack of a readily available diagnostic test have contributed to a high prevalence of disease. The sequelae of undiagnosed and untreated infection have become an important health problem for men and women. Some proven and suspected complications of untreated chlamydial infection include epididymitis, pelvic inflammatory disease, sterility, ectopic pregnancy, and perihepatitis (7, 12). Infants born to mothers with chlamydial cervicitis are at risk for inclusion conjunctivitis and chlamydial pneumonia (8).

A recently developed technique with a fluorescein-conjugated monoclonal antibody to detect antigens of *C. trachomatis* offers promise of a rapid and accurate method of diagnosis (15). However, prevention of disease through the use of effective prophylactic agents would have greater impact on the prevalence and control of disease, if such prophylaxis would be used widely.

This study demonstrates that spermicides that contain N-9 have marked antichlamydial activity when assayed in an in vitro system. This study does not demonstrate the exact mechanism of this activity. Determining whether N-9 is chlamydiacidal or only interferes with attachment or penetration into the target cell will require further study. The in vitro activity of commercially available spermicides against many sexually transmitted agents, including *N. gonorrhoeae*, *Trichomonas vaginalis*, *Candida albicans*, *Treponema pallidum*, and herpes simplex virus, has been documented (2, 3, 5, 13, 14). In one clinical study, phenylmercuric acetate was found to be slightly more effective than N-9 in reducing gonorrheal incidence in a cohort of women (10). Other clinical studies, both retrospective and prospective, suggest that the use of spermicides by sexually active women may reduce their risk of gonorrhea (1, 4, 6). No clinical studies have addressed the effect of spermicides on the risk of chlamydial infection, and few, if any, in vitro data are available on the activity of spermicides against *C. trachomatis*. The results of this in vitro study suggest significant clinical potential for spermicides in the preven-

tion of infections due to *C. trachomatis*. Further evaluation through the performance of clinical trials seems warranted.

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